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A new species of *Arrhenia* (Agaricales, Hygrophoraceae) from Guizhou Province, China

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Abstract: *Arrhenia nivea* is a new bryophilous species of *Arrhenia* from a valley in southwestern China's subtropical monsoon humid climate zone. The species is characterized by pure white basidiomata with an irregularly discoid to cupulate 1.5–4.5 mm diameter pileus, reduced hymenium, nonamyloid basidiospores, lack of cystidia, the presence of clamps, and no incrusting pigment of the pileipellis hyphae. Phylogenetic analysis using ITS sequence data confirmed that it arised from a pathway separate from other species of *Arrhenia*.

Key words: Morphology, moss, phylogeny, taxonomy

1. Introduction

Arrhenia Fr. was erected by Fries (1849) for three bryophilous species, of which only the type species, *A. auriscalpium* (Fr.) Fr., remains. The genus gained many pleuroteloid species after synonymization with the reduced hymenial *Leptoglossum* P. Karst (Kühner and Lamoure, 1972) and the lamellate *Phaeotellus* Kühner & Lamoure (Kühner, 1980). The DNA era ushered an influx of omphalinoid species, formerly included in *Omphalina* Qué. (Redhead et al., 2002). The result is a pleomorphic genus with species of omphalinoid or pleuroteloid to cyphelloid habitus, with or without a stipe, and a lamellate to variously reduced hymenium. Most basidiomata are brownish grey, occasionally very light, but no truly white species is known; even the near-white *A. eburnea* Barrasa & V.J. Rico was described with some incrusting pigment of the pileipellis (Barrasa and Rico, 2003). Other micromorphological characteristics, common to most species, include nonamyloid basidiospores, absent cystidia, subregular to irregular hymenophoral trama, and the presence of clamp connections (Redhead et al., 2002; Lodge et al., 2013). Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>, last accessed March 2022) lists 83 taxa, 78 species, and 5 varieties, with worldwide distribution; most species have been described from temperate regions. The genus is believed to have

a mutualistic relationship with bryophytes, although several members of the *A. acerosa* complex were noted to have a saprobic relationship with dead plant matter (Voitk et al., 2020). *Arrhenia* was placed in Hygrophoraceae by Lodge et al. (2013), but an encompassing phylogenetic analysis of the genus has not been done.

During recent surveys of macrofungi in Tongren City (Jiangkou County, famous for its typical karst landscape) in Guizhou Province, China, we found several unusual small white bryophilous *Arrhenia*-like fungi from moss. Based on morphological descriptions and molecular analyses, our specimens represent a new species.

2. Materials and methods

2.1. Collection and morphologic examination

The specimens were collected growing on moist soil among short mosses from the Yamugou Scenic Area, Tongren City, Guizhou Province, China. The site is adjacent to Fanjing Mountain, approximately 500 m above sea level, in a subtropical humid climate zone. Standard field techniques were used and macromorphology was noted from fresh material in situ. Colour codes of fruiting bodies are from HTML Colour Codes (<https://htmlcolorcodes.com>). Specimens were dried at 40 °C and deposited in the Herbarium of Guizhou Institute of Biology, China (HGASMF). Preparations in 1% Congo

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Red and 5% KOH were made from dried specimens for microscopy, using a Nikon H550S light microscope up to 1000 × magnification. Basidiospore measurements follow Zhang et al. (2020). Microscopic structures were drawn under a drawtube.

2.2. Molecular phylogeny

2.2.1. DNA extraction, amplification and sequencing

DNA extraction followed Kuhad et al. (2004), and the nrITS region amplified as reported by White et al. (1990). PCR was performed using 1.5 µL template DNA, 0.5 µL of each primer, 10 µL distilled water, and 12.5 µL PCR mix. Thermocycler conditions were performed according to Lodge et al. (2013). PCR products were purified and sequenced by Sangon Biotech (Shanghai, China).

2.2.2. Phylogenetic analysis

BLAST search in the NCBI GenBank dataset, using the nrITS sequence of the new species yielded the highest similarity, 91.59%, to OM456379 from the USA. For the analysis, we chose 34 GenBank nrITS sequences of *Arrhenia* species with similarity greater than 85% (Table). Sequences were aligned using MAFFT version 7 (<https://mafft.cbrc.jp/alignment/software/>) with default settings (Latha et al., 2018). Analysis datasets were examined using Bayesian inference (BI; Ronquist and Huelsenbeck, 2003) and maximum likelihood (ML; Nylander, 2004). The maximum likelihood tree was searched in FigTree v1.3.1, and Bayesian tree data were added to the corresponding nodes.

3. Results

3.1. Taxonomy

Arrhenia nivea Chun Y. Deng & Huan Gao sp. nov. (Figures 1, 2, and 3).

Mycobank no: MB 838932.

Diagnosis:—Small bryophilic cyphelloid species distinguished from other arrhenias by its pure white color, lack of incrusted pigment, location in subtropical China, and ITS data.

Etymology:—The epithet *nivea* (snowy) refers to the white colour of the basidiomata.

Typification:—CHINA. Guizhou Province, Tongren City, Jiangkou County, Taiping Town, Zhaibao Village, Yamugou Scenic Area, 27°47'12" N, 108°43'50" E, alt. 478 m, 21 April 2020. (HGASMF01-13011, holotype).

Description:—*Basidiomata* (Figure 1) 1.5–4.5 mm diam., astipitate, smooth, fragile, thin-walled discs, at least bilaterally symmetrical, margin becoming crinkled or undulate, rarely with a lateral cleft; projecting from an occasionally slightly rugulose pileipellic attachment to moss in a near-plane through cyphelloid to frankly cupulate form, exposing the smooth concave hymenial surface, inferiorly, superiorly, or laterally; translucent,

pileipellis and hymenium entirely pure white (FFFFFF) to cream (F5FFFA). Context very thin, whitish, immutable when exposed. Taste and odor indistinct. *Basidiospores* (38/6/60) 6.0–8.5 (9) × 3.0–5.0 µm, $L_m = 7.04 \pm 0.85$ µm, $W_m = 4.21 \pm 0.49$ µm, $Q = 1.30\text{--}2.00$ (2.50), $Q_m = 1.69 \pm 0.25$, lacrymoid to pipshaped, ovoid, or broadly elliptical, with a very prominent apiculus, smooth, hyaline, nonamyloid (Figure 2). *Basidia* 25.5–33.5 × 5.5–8.5 µm, subcylindrical to clavate, elongated, 2–4 spored, sterigmata 2.8–5.0 µm long. *Basidioles* 20.0–31.0 × 4.2–8.3 µm, subcylindrical to clavate. *Cystidia* absent. *Pileipellis* a cutis of subparallel to interwoven hyphae, irregularly distributed, terminal cells obtuse, 12.5–44.5 µm long, 3.0–7.0 µm wide; no convincing evidence of incrusting pigment found (Figure 3). *Tramal hyphae* interwoven, 3.8–9.0 µm wide, smooth, nonamyloid, nongelatinous, clamped, with thin to pronounced walls.

Habitat:—scattered in gregarious groups on mosses growing on rock, mulch, and duff; in the spring.

Additional specimens examined:—CHINA. Guizhou Province, Tongren City, Jiangkou County, Taiping Town, Zhaibao Village, Yamugou Scenic Area, 27°47'12"N, 108°43'50" E, alt. 456 m, 26 May 2020. 520621MF410 (HGASMF01-8234, paratype).

3.2. Molecular phylogeny

The pathway to *Arrhenia* was resolved with ML-BP 98% and BI-PP 1.00 support, relative to the chosen outgroups (Figure 4). Of the specimens analysed, *A. gerardiana* (pathway C) was the most ancestral. Its sister pathway bifurcated, one arm (pathway B) leading to omphalinoid species, and its sister (pathway A) led to a mixture of stipitate and astipitate lamellate and alamellate species. *Arrhenia nivea* arose as a terminal clade from one long arm with ML-BP 100% and BI-PP 1.00 support; all the other species from pathway A arose from an arm sister to *A. nivea*. The ITS dataset of this new *Arrhenia* species had an alignment length of 826 nucleotide positions (including gaps), and all sequences cover ITS-1, 5.8S, and ITS-2 regions.

4. Discussion

These days, when most new species are cryptic members of a complex, uncovered by phylogenetic analysis, it is a delight to find a beautiful and clearly distinct new species. The pileus of most species of *Arrhenia* is greyish, blackish, bluish or brown-grey (Redhead et al., 2002), and can be clearly distinguished from *A. nivea*. Although there are some near-white species of *Arrhenia*, e.g., *A. eburnea* Barrasa & V.J. Rico (Barrasa et al., 2003), and some whitish members of the *Arrhenia acerosa* complex (Voitk et al., 2020) their well-developed gills, and often at least a rudimentary stipe, prevent them from being mistaken for *A. nivea*. Of white cyphelloid species from

Table. Voucher information and GenBank accession numbers of specimens included in the molecular phylogenetic analyses of this study.

Species	Voucher	Locality	GenBank accession numbers (ITS)	Reference
<i>Arrhenia nivea</i>	HGASMF01-13011	China	MW491461	This study
<i>Arrhenia nivea</i>	HGASMF01-8234	China	MW491462	This study
<i>Arrhenia acerosa</i>	TU109632	Estonia	MT967311	Voitk et al. (2020)
<i>Arrhenia acerosa</i>	O189467	Norway	MT967310	Voitk et al. (2020)
<i>Arrhenia auriscalpium</i>	Lutzoni 930731-3, DUKE	Iceland	U66428	Lutzoni (1997)
<i>Arrhenia chlorocyanea</i>	iNat 20526963	USA	MN202585	GenBank
<i>Arrhenia elegans</i>	MCVE-16455	Italy	JF908757	Osmundson et al. (2013)
<i>Arrhenia elegans</i>	WU_36410	Austria	MW113699	GenBank
<i>Arrhenia epichysium</i>	LE262961	Russia	KC237880	Zvyagina et al. (2015)
<i>Arrhenia epichysium</i>	17148	Finland	JF908504	Osmundson et al. (2013)
<i>Arrhenia gerardiana</i>	DAOM744373	Canada	MH473366	GenBank
<i>Arrhenia gerardiana</i>	DAOM744374	Canada	MH473361	GenBank
<i>Arrhenia gerardiana</i>	TU117592	Canada	MH198226	GenBank
<i>Arrhenia latispora</i>	BBF-GC01082301	France	MT967323	Voitk et al. (2020)
<i>Arrhenia lobata</i>	O73053	Svalbard	GU234033	Geml et al. (2012)
<i>Arrhenia lobata</i>	Lutzoni & Lamoure 910825, DUKE	France	U66429	Lutzoni (1997)
<i>Arrhenia obscurata</i>	MON3957	Canada	MT998930	Voitk et al. (2020)
<i>Arrhenia obscurata</i>	MON3292	Canada	MT998924	Voitk et al. (2020)
<i>Arrhenia obscurata</i>	MON3334	Canada	MT998927	Voitk et al. (2020)
<i>Arrhenia peltigerina</i>	TU-117595	Canada	MT967339	Voitk et al. (2020)
<i>Arrhenia peltigerina</i>	TU-117465	Canada	MT967337	Voitk et al. (2020)
<i>Arrhenia philonotis</i>	DAOM744402	Norway	MH473368	GenBank
<i>Arrhenia philonotis</i>	Lutzoni 930804-5, DUKE & O	Iceland	U66449	Lutzoni (1997)
<i>Arrhenia retiruga</i>	TU-117227	Estonia	MT967340	Voitk et al. (2020)
<i>Arrhenia retiruga</i>	GB-0150441	Sweden	EU118604	Larsson (2007)
<i>Arrhenia salina</i>	O-260200	Svalbard	MT967341	Voitk et al. (2020)
<i>Arrhenia spathulata</i>	TU-105605	Estonia	MT967347	Voitk et al. (2020)
<i>Arrhenia spathulata</i>	TAAM-172613	Estonia	MT967343	Voitk et al. (2020)
<i>Arrhenia subglobisemen</i>	MON3333	Canada	MT998926	Voitk et al. (2020)
<i>Arrhenia subglobisemen</i>	DAOM-981255	Canada	MT998922	Voitk et al. (2020)
<i>Arrhenia svalbardensis</i>	O-50444	Svalbard	MT967357	Voitk et al. (2020)
<i>Arrhenia telmatiaea</i>	NY12555	United Kingdom	OM422834	Voitk et al (2022)
<i>Arrhenia telmatiaea</i>	GB-0065942	Sweden	MH198228	Voitk et al (2022)
<i>Arrhenia telmatiaea</i>	M-0276632	Germany	MH198193	Voitk et al (2022)
<i>Rimbachia bryophila</i>	MushroomObserver.org/445933	USA	OK485913	GenBank
<i>Rimbachia bryophila</i>	MushroomObserver.org/364492	USA	MW871710	GenBank
<i>Rimbachia bryophila</i>	Mushroom Observer # 265975	USA	MH567099	GenBank
<i>Rimbachia arachnoidea</i>	K(M):237151	United Kingdom	MZ159675	GenBank

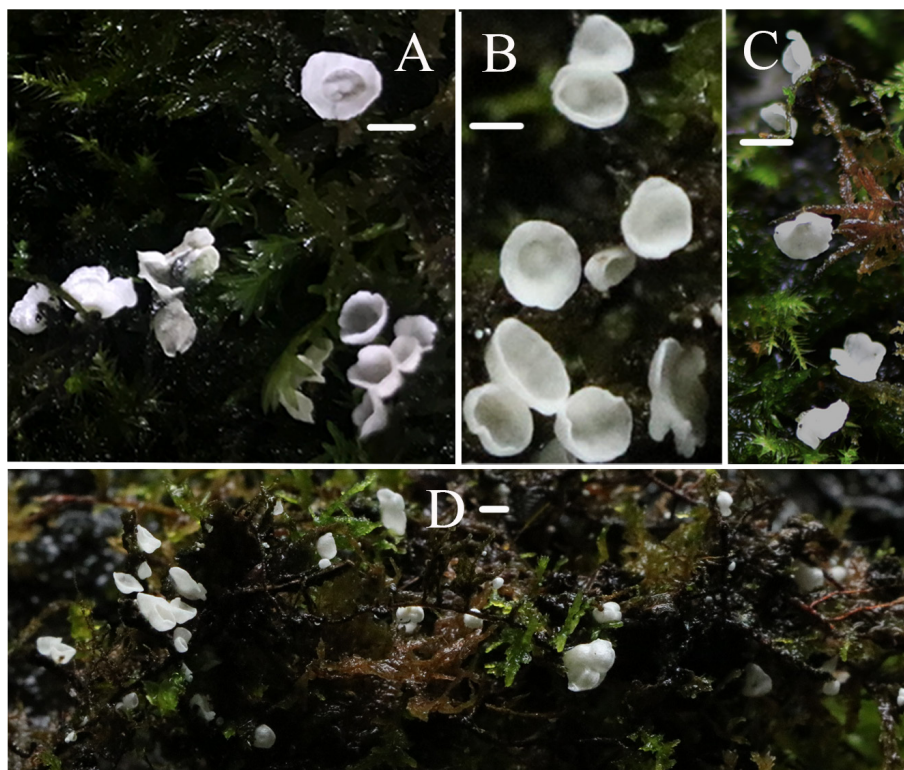


Figure 1. Basidiomata of *Arrhenia nivea* in its natural habitat. A, D: HGASMF01-13011. B, C: HGASMF01-8234, Paratype. Bar: 2 cm.

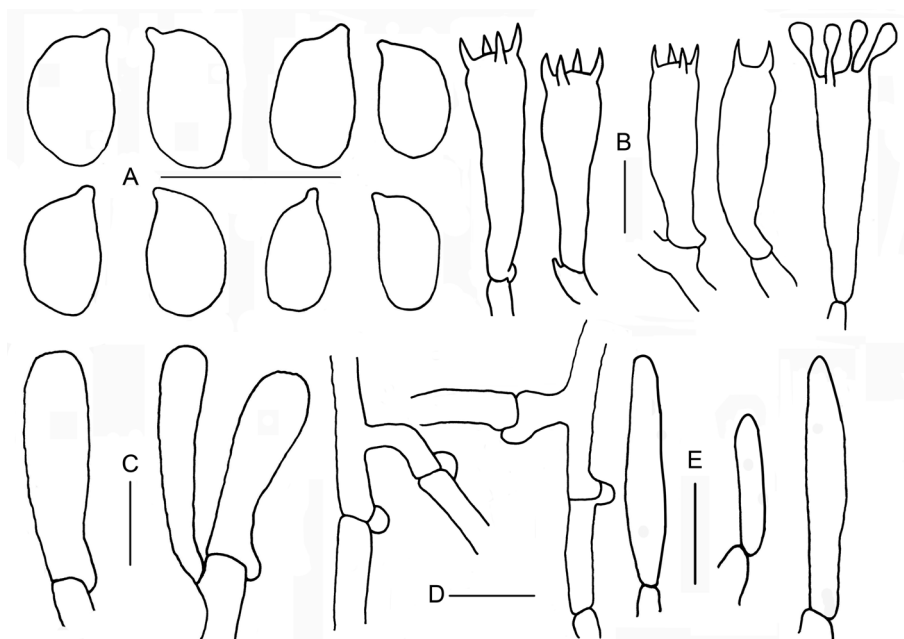


Figure 2. Microstructures of *Arrhenia nivea* (holotype, HGASMF01-13011). A: Basidiospores. B: Basidia. C: Basidioles. D: Clamp connection. E: Pileipellis hyphal ends. Bars: 10 μ m.

other genera, *Cellypha goldbachii* cannot be confused because of its substrate of herbaceous stems; it also differs by having clavate hairs on the pileus, longer

basidiospores, and hymenial cystidia (Uzun et al., 2018). One similar bryophilous species, *Rimbachia bryophila* (Pers.) Redhead, has smaller basidiospores (5–7 \times 4.5–7

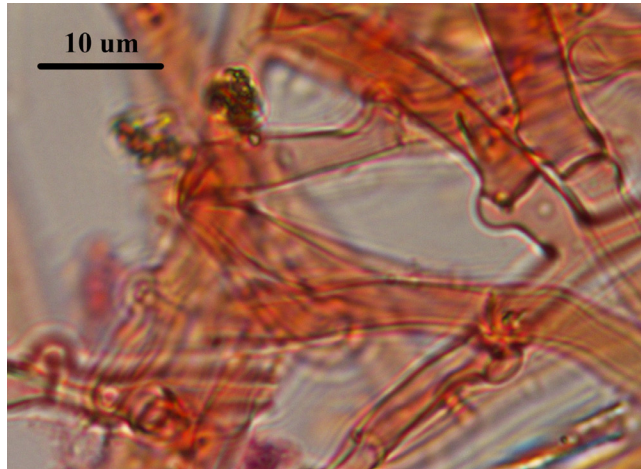


Figure 3. Hyphae of the pileipellis showing clamp connections Zebra bars characteristic of incrusted pigment not seen. Bars: 10 μ m.

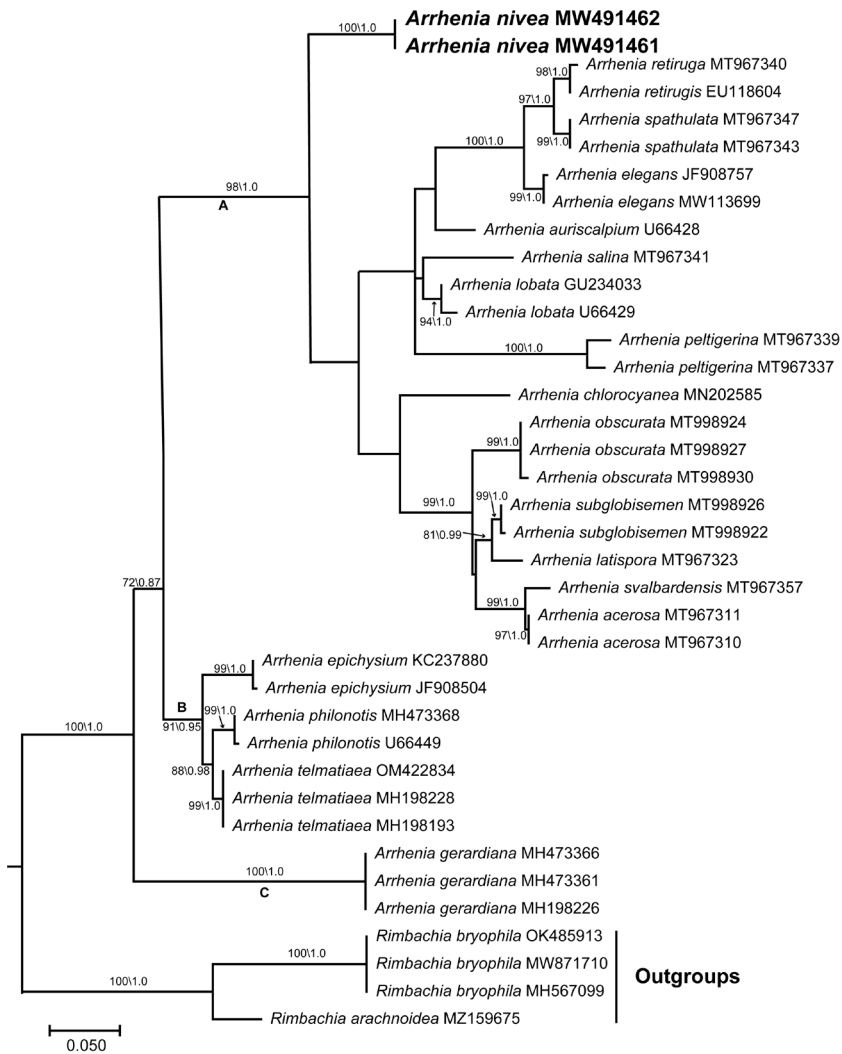


Figure 4. Maximum likelihood (ML) and Bayesian inference (BI) phylogeny of *Arrhenia* based on nrITS sequences. ML-BS \geq 70% and BI-PP \geq 0.85 are shown on the branch. Values to the right and left of the '/' are ML-BS and BI-PP, respectively. GenBank accession numbers are indicated after the taxonomic names.

µm) and basidia (19–25 × 6–9) than *A. nivea* (Redhead, 1984), and another, the minute *Mniopetalum miniatum* M. Zang, has ten times smaller basidiomata 0.3–0.5 mm diam. (Zang, 1988). Of cyphellate-cupulate arrhenias, only the basidiomata of *A. retiruga* are about the same size and shape, and often (not always) also have a significantly reduced hymenium. However, even if dried very light, they remain somewhat straw coloured, which changes to yellowish grey or hazel grey on rehydration; also, they lack clamp connections in all tissues (Barrasa and Rico, 2003). A major difference between this species and all other arrhenias is its location and habitat. *Arrhenia nivea* was discovered closer to the Tropic of Cancer than most species of the genus and is native to subtropical China.

5. Conclusion

Arrhenia nivea is a unique species of *Arrhenia*, identified by morphology, distribution and habitat preference. It is the first truly white species of *Arrhenia*; phylogenetic analysis shows that it is a distinct species, not an albino form of a known species.

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